

ANAPHOIDEA CALENDRÆ GAHAN, A MYMARID
PARASITE OF EGGS OF WEEVILS OF
THE GENUS CALENDRA¹

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ABSTRACT

The corn billbug egg parasite, *Anaphoidea calendræ*, is a monembryonic, gregarious, hymenopterous insect of the family Mymaridæ, parasitizing eggs of several species of *Calendra*, especially *C. minima*, *C. parvula* and *C. callosa*, all of which hosts are very destructive to corn, small grains and grasses. A usual number of parasites developing in one host-egg is seven—one male and six females. The species is able to perpetuate itself in the eggs of numerous additional species of beetles of this genus, and occasionally it matures three times the usual number, especially when parasitizing the eggs of the larger species. Its known distribution is such as to indicate that it is established throughout the eastern half of the United States. It has been found to destroy seventy-five per cent. of the billbug eggs in *Agrostis alba*, in which host plant of *Calendra* it has been most intensively studied. In the latitude of St. Louis, Mo., there are

¹ Gahan, A. B., 1927. Miscellaneous Descriptions of New Parasitic Hymenoptera with some Synonymical Notes. Proc. U. S. Nat. Mus., Vol. 71, Art. 4, pp. 1-39 (31-35).

² The writer wishes to acknowledge valuable inspiration in the study of this mymarid from Dr. L. O. Howard and Charles C. Hill; indebtedness to Mr. A. B. Gahan, who described this species and generously lauded the work; and indispensable assistance from the laboratory staff, R. C. Lange and Hervey E. Roberts, in securing the necessary *Calendra* eggs. Especial appreciation is due Miss Frances H. Pickel for watching and aiding in recording the behavior of these tiny adults, and to Alexander F. Bucholtz for the dissections in the study of living eggs and young larvæ. Particular thanks are due to Professor R. W. Harned, A. and M. College, Miss., through whose generous cooperation with the Bureau of Entomology the first authentic *Anaphoidea calendræ* were taken.

at least four generations annually, sometimes probably seven generations. The larvæ remain inactive from July, August, or September, pupating in the following spring.

INTRODUCTION

Anaphoidea calendræ, the only recognized internal insect parasite in the eggs of species of *Calendra* (*Curculionidæ*), was established as a new species by Mr. A. B. Gahan, from material collected by the writer in Mississippi and Missouri in 1922 and subsequently.³ It has responded well, under laboratory conditions, to breeding through numerous species of the host genus and has attracted the attention of the Hawaiian Sugar Planters' Association as a possibly adaptable parasite for introduction into Hawaii for the control of the sugarcane borer, *Rhabdocnemis obscurus*. This paper summarizes the work done on this species, except that done for the Hawaiian Sugar Planters' Association by Dr. F. X. Williams.

GEOGRAPHICAL DISTRIBUTION

The first positive record of distribution of this interesting parasite was obtained by rearing it from a *Calendra* egg collected at Greenville, Washington County, Miss., June 15, 1922, and from two eggs collected at Grenada, Grenada County, Miss., the following day, all of which issued at the laboratory on June 23.

The next locality yielding this parasite was Boschertown, St. Charles County, Mo., issuing July 10, 1922.

Some *Calendra* eggs gathered from the laboratory plats at Webster Groves, St. Louis County, Mo., for parasitization by the *Anaphoidea calendræ*, yielded parasites, some on July 10, 1922, the day of the collection.

Other localities from which this parasite has been collected are Grover and Kirkwood, St. Louis County, and Rocheport, Boone

³ The writer narrowly missed discovering this species in 1915, when he observed two eggs to be developing spots and therefore discarded them as spoiling. Two tiny hymenopterous insects were also recovered from a cage in which eight eggs had been placed on July 15, 1915, from timothy at West Lafayette, Ind.

County, Mo., Elberon, Monmouth County, N. J., and, beyond a reasonable doubt, West Lafayette, Tippecanoe County, Ind.

THE HOST-PLANTS AND EGGS OF CALENDRA

Parasitized eggs of the several species of the genus *Calendra* are obviously not determinable by rearing. Furthermore, approximate determinations only can be made by correlating the host-plant, the host-egg, and the locality, aided imperfectly by measurements of the egg.

The host-plant of *Calendra* at Greenville, Miss., was *Juncus torreyi*. Eggs from this plant collection yielded one adult *Calendra callosa* and one adult *C. destructor*.

The *Calendra* host-plant at Grenada, Miss., was *Juncus acuminatus*, which contained, in one of the two collections yielding the parasite, one egg which proved to be *C. destructor*.

The plant at Grover, Mo., in each collection, was *Agrostis alba*. In a large series of *Calendra* eggs collected July 17, 1925, sixteen proved by rearings to be those of *C. minima*, and two, of *C. parvula*.

The plants at Kirkwood, Mo., were timothy and *A. alba*, chiefly the latter. *Calendra parvula* and *C. minima* infest each plant. *C. parvula* is dominant in timothy, *C. minima* in *A. alba*.

The plant at Rocheport, Mo., was *Cyperus esculentus*, which yielded *Calendra* eggs from which *C. callosa* was reared.

The plant at Boschertown, Mo., was terrell-grass, *Elymus virginicus*, which yielded a *Calendra* egg which proved to be that of *C. minima*, apparently the only species of the genus *Calendra* breeding in it.

The plants at Webster Groves, Mo., were timothy, *A. alba*, and *C. esculentus*, and it is inferred that the hosts of the parasites in this locality are *C. parvula*, *C. minima*, and *C. callosa*.

The *Calendra* host-plant at Elberon, N. J., was timothy and the host-egg probably that of *C. parvula*.

As *Anaphoidea calendrae* parasitizes one or more of these three very common species of the genus *Calendra* in Mississippi, Missouri, and New Jersey, it may be expected to occur generally where these hosts occur, from South Dakota to Texas and east to the Atlantic Coast.

ECONOMIC IMPORTANCE OF ANAPHOIDEA CALENDRAE

Of the seventy-five or more known species of the genus *Calendra* occurring in the United States, all are potential destroyers of corn and small grains and grasses, all of them in the adult stage and many in the larval stage. Damage by them to lawns, golf courses, and pastures is particularly difficult to appraise. It seems fairly certain from my observations, that every sod that is two years old or older is substantially infested. Damage to timothy may be easily demonstrated by lifting bunches of this grass while the ground is dry in summer and observing the percentage of the corms that have been excavated or that have the bases of the corms and the roots completely severed from the stalk. The failure of the timothy to be profitable for harvest in its second or third year may be attributed largely to the work of species of this genus. The manner of growth and size of stems of bluegrass, Bermuda grass, and the bent grasses, all of which are subject to heavy infestation, make it much more difficult to perceive in them than in timothy the havoc wrought by these weevils.

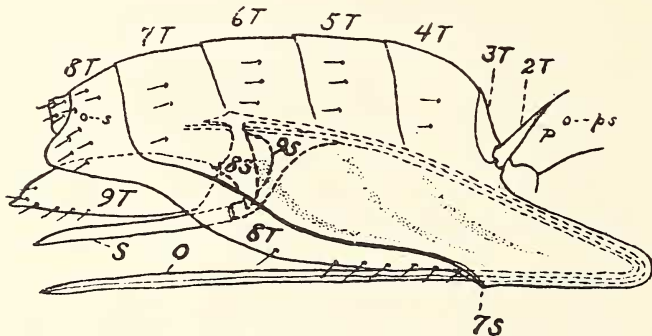


FIG. 1.—*Anaphoidea calendrae* Gahan. Diagram of female abdomen. P, propodeum; ps, Propodeal spiracle; 2T, Second tergite; 3T, Third tergite; 4T, Fourth tergite; 5T, Fifth tergite; 6T, Sixth tergite; 7T, Seventh tergite; 8T, Eighth tergite; 9T, Ninth tergite; O, Ovipositor; s, spiracle; S, Ovipositor sheath; 7S, Seventh sternite; 8S, Eighth sternite; 9S, Ninth sternite. (After Gahan, A. B., Bureau of Entomology, U. S. Dept. of Agriculture. Proc. of the U. S. N. M. Vol. 71, Art. 4, p. 32.)

The economic importance of *Anaphoidea calendrae* as a control of these destructive corn billbugs is best expressed in ob-

served percentages of infestation found in field examinations. Occasional small collections of one to five eggs each were 100 per cent. infested. Extensive collections were made at two places, one near the field station in Webster Groves, the other about twenty-five miles west, near Grover, Mo. At the latter place one collection of twenty-one eggs, June 22, yielded one parasitized egg, 4.8 per cent., while a second collection of ninety-eight eggs, July 17, yielded thirty parasitized eggs, 30.6 per cent. The evident difference is attributable to the stepping-up of efficiency as the broods progress. At Webster Groves a succession of collections was made for seven days, beginning July 8, ending July 16. One hundred and twelve eggs were collected, yielding eighty-five parasitized eggs, 75.4 per cent.

This parasite attacks the host egg and destroys it at the point of oviposition.

The larvæ are gregarious, usually about seven, in the ratio of one male to six females, in each egg.

The life cycle is short, usually complete in fourteen to seventeen days in warm weather.

The breeding season is long, admitting, apparently, of seven broods a year, in the St. Louis latitude.

The parasite is known to attack three very common, seriously destructive species of billbugs and, in control, adapts itself readily to numerous other species.

It is widely distributed, being known to occur in the Gulf region, on the Atlantic Seaboard and in the central Mississippi River Basin.

HAWAIIAN INTEREST IN ANAPHOIDEA CALENDRE

Mr. C. E. Pemberton, on behalf of the Hawaiian Sugar Planters' Association, Honolulu, T. H., began correspondence with the writer, July 11, 1927, after reading Mr. Gahan's notes following his description of the new species. He stated that the sugarcane borer, (*Sphenophorus*) *Rhabdocnemis obscurus*, has been a major problem in their cane fields, in Hawaii, for over forty years and still does considerable damage in spite of one successful introduction of a parasite by Mr. Muir in 1910.

Mr. Pemberton spent two and a half years in the Philippines, Java, Borneo, Celebes, and other islands of the Malay Archipelago in search of natural enemies of this weevil. Though he found and shipped seven or eight predatory or parasitic insects, and collected quantities of eggs of *Rhabdocnemis*, *Calendra*, *Diocalandra*, *Sphenophorus*, and *Rhynchophorus*, and Mr. Muir had made collections in New Guinea, Java, Amboina, and other places between 1906 and 1910, neither investigator had found an egg parasite of any beetle belonging to these genera. The records of *A. calendrae* were therefore of particular interest.

Mr. Pemberton stated that *Rhabdocnemis* imbeds its eggs in the sugarcane stalk, and inquired into the oviposition habits of *Calendra*, also concerning the sizes of eggs, seasons of activity, the possibility of accumulating a supply of parasitized eggs and of shipping them.

The outcome of this and further correspondence was that the Hawaiian Sugar Planters' Association decided that there was enough chance of a profitable introduction of this mymarid egg parasite into the sugar plantations of Hawaii, to make the attempt in 1928. The work was entrusted to Dr. Francis X. Williams, of the Sugar Planters' Experiment Station Staff, who arrived at the Webster Groves laboratory May 10, and with the cooperation of the Bureau of Entomology at this laboratory, assembled a mass of material and carried this to Hawaii in person, without issuance en route. Dr. Williams left the laboratory July 21, which was one generation earlier than the hibernating generation.

He reported that emergence was satisfactorily accomplished, but evidence of the parasites attacking eggs of *Rhabdocnemis obscurus* was lacking.

REARINGS FROM EGGS PARASITIZED IN NATURE AND IN CONTROL

The number of adults maturing from *Calendra* eggs parasitized before they were collected and parasitized under control are given in the accompanying table (Table 1), first the number of males, then females, then the number of sexually undetermined under an interrogation mark, with an asterisk where not all of the matured adults issued.

As the *C. ? minima* collection of eggs is the outstanding series of supposedly one species, it will be well to study first the numbers per host egg and ratios of sexes in this species. One egg produced 3 adults; 2 produced 4; 6 produced 5; 21 produced 6; 34 produced 7; 10 produced 8; 4 produced 9; 1, 10; 2, 11; and 1, 12. Seven adults per host egg is much more frequent than any other number, six per host egg is next. Possibly the higher numbers may indicate that some eggs in the series are those of a larger species, *C. parvula*, for instance.

In the matter of sex ratios, 12 were without a male, 58 had one male each, 8 had 2 each, 3 had 3 each, and 1 had 6 males and no females, the only host egg in 82 from nature maturing males only. Normally, both sexes are present. However, four parasite groups out of six groups brought to maturity from one of the first three groups used for propagation yielded males only, as if the species were arrhenotokous and not all the females in the parent group had mated. Subsequently, no eggs were submitted for parasitism until matings were observed.

Only one *C. minima* egg of known parent was parasitized. The number of *A. calendrae* developing in it was seven, the number which occurred with greatest frequency in the large series of supposed *minima*.

The records of parasitism of eggs of species of *Calendra* larger than *C. minima* indicate that the sex ratios in all species are essentially as in *C. minima*, and that more than the normal lot of eggs are deposited in some large host-eggs.

It is conceivable that such irregularity may occur alike in the several host-egg species, but that only in the host-eggs of larger content will the increased number of progeny reach maturity, while those in host eggs of lesser capacity will perish by starvation, or the number reaching maturity may be reduced by cannibalism.

Occasionally parasitism took place with results fatal both to the host and to the parasites.

NUMBER OF GENERATIONS

It may be expected that the number of generations annually is variable in each locality, partly owing to a normal overlapping

TABLE I

RECORDS OF NUMBERS OF THE RESPECTIVE SEXES MATURING FROM *Calendra* EGGS OF GIVEN SPECIES. (THE COLUMN UNDER THE QUESTION MARK REPRESENTS ADULTS COUNTED BUT LOST WITHOUT SEX BEING RECORDED. THE ASTERISK MARKS THE GROUPS IN WHICH NOT ALL ADULTS ISSUED FROM THE HOST-EGG.)

Host spp.	Number of parasites			No. eggs	Number of parasites			No. eggs	Number of parasites			No. eggs
	♂	♀	?		♂	♀	?		♂	♀	?	
<i>C. ?minima</i>	0	3*		1	0	5		1	0	5*		1
	0	5	1	1	0	6		2	0	6*		1
	0	6	1	1	0	7		2	1	3		2
	1	4		1	1	5		10	1	5*		4
	1	6		23	1	6*		5	1	7		5
	1	7*		4	1	8		1	1	9		1
	1	4	2	1	1	6	1	1	2	4		1
	2	4*		1	2	5		1	2	7*		3
	2	9		1	2	10		1	3	2		1
	3	3	1	1	3	7		1	6	0		1
	1	5	1	1								
	1	5	2	1	1	1	1	1			7	1
	7		1	1								
<i>C. minima...</i>	1	2		1	1	5		1	1	7		1
<i>C. ?callosa...</i>	1	9		1	1	10		1	8	0		1 ¹
<i>C. callosa ...</i>	9	0		1 ¹	10	0		1 ¹	22	1		1 ¹

¹ These four eggs were parasitized by a single group of adults.

TABLE I (Continued)

Host spp.	Number of parasites			No. eggs	Number of parasites			No. eggs	Number of parasites			No. eggs
	♂	♀	?		♂	♀	?		♂	♀	?	
<i>C. ?destructor</i>		1	10	1			9	1			13	1
	1	8		1								
<i>C. destructor</i>	0	1*		1	0	12	4	1	1	6		1
	1	7		1	3	6		1	5	2		1
	8	0		1	13	1		1				
<i>C. ?parvula</i>	0	5	1	1	1	4		1	1	5		1
	6	2		1								
<i>C. parvula...</i>			10	1			13	1	1	9		1
	2	8		1								
<i>C. ?perinax</i>			10	1								
<i>C. pertinax</i>	0	8		1	1	4		1	1	5		1
	1	6		1	1	7		1	2	19		1
<i>C. costipennis</i>		9	4	1	1	10		1	1	11		1
	1	12		1								
<i>C. maidis...</i>	0	9		1	1	7		1				

of broods, partly to a variation in speed of development in the several hosts, partly to temperature fluctuations and partly to latitude.

Eggs of the several species of *Calendra* believed to be hosts of *Anaphoidea calendra* in the Webster Groves, Mo., locality have been taken occasionally as early as May 1, and as late as September 3.

The records of eight series of rearings of *A. calendra* including the issuance of thirty-five groups, are given on the accompanying chart (Fig. 2). The dates of collection are represented by the left termini of the solid lines. The dotted extensions of these lines indicate the unknown beginnings for the respective series. All records are from Missouri, in the latitude of St. Louis. The date of issuance of each group of adults is indicated by a colon to the right of the host-species name.

Those lines projected to the right without a terminating colon represent groups whose larvæ continued into winter.

The dates are shown at top and bottom of the chart, and all groups issuing in one day are in a vertical line.

No series was continued through the complete year. Three of the series observed show conclusively that there may be at least four generations annually. Each series starts with an egg deposited during the then current season and completes two more generations during the year.

The first entry made is that of a group of *Anaphoidea calendra* within a *Calendra ? minima* egg collected June 5; the egg had been deposited shortly before, and hence this was not a group of hibernating parasites. This group may therefore represent the eggs of the first or of the second brood of parasites, and might very doubtfully be of the third brood. In case it belonged to the first brood, we may assume that the hibernating brood of parasites issues in this region about June 3, the first brood about June 19, the second brood about July 3, the third about July 16, the fourth about July 30, the fifth about August 15, the sixth about September 1, and that suspension of development of larvæ occurs in an indeterminate fraction of the host-egg groups in the fourth, fifth, sixth, and seventh broods, but chiefly in the sixth.

It is not to be expected that all groups of adults from the hibernating brood would issue simultaneously. Probably the

bulk of them would issue in any given locality within a period of ten days. Each group should live in the adult state for three or more days, and oviposit throughout that time. In the duration of the life cycle of individual groups there is a variation of from 13 to 18 days (38 per cent.) in *destructor* as host, 14 to 18 days (28 per cent.) in *parvula*, and 14 to 26 days (85 per cent.) in *costipennis*. It thus becomes apparent that no vertical column in this chart can be considered narrowly to define any one brood.

According to records made by the writer, the summer broods of *A. calendrae* developed as follows: In eggs of *C. parvula*, two eggs, 14 and 18 days, an average of 16 days; in eggs of *C. minima*, one egg, 18 days; in eggs of *C. costipennis*, three eggs, 14, 17, and 26 days, an average of 19 days; in eggs of *C. pertinax*, five eggs, 14 to 17 days, average 15 days; in eggs of *C. maidis*, two eggs, 17 and 18 days, average, 17.5 days; in eggs of *C. destructor*, seven eggs, 13 to 18 days, average, 15.2 days. These twenty-nine groups required an average of 16.6 days between the issuance of the parent adults and the progeny adults. Development in eggs of *destructor* occurred more quickly than in those of *callosa*, as shown by groups of common parentage and date. Opportunity for observation of development in other species was not so favorable by reason of the smaller numbers under investigation.

BEHAVIOR OF ADULTS

The behavior of the adult *Anaphoidea calendrae* is quite varied, often ludicrous.

When ready to issue from the host egg, they bite out pieces of the amnion and chorion till they have made one or more approximately circular exits. As the adults issue, they run swiftly and erratically or deliberately preen their antennæ, wings, and feet. They are proficient at jumping. Often they feigned death, as the result of collision with another adult or with the walls of the cage. After feigning death, they would rise to their feet and proceed as if nothing had happened. Occasionally they were observed to fly within the cage. When one escaped to the desk top and took flight, it was visible when the light hit its wings favorably. The flight was jerky, more or less spiral, and van-

ishing. Most flights were very short, so that the attendant watched for it to alight within three or four inches from the starting point, prepared to place an inverted culture tube over it for its recapture.

Heliotropism among the adults is sufficiently pronounced to aid substantially in handling them, as discussed under "Methods."

There appears to be no definite rule for priority of issuance of the sexes. Very promptly after issuance, the male seeks to find and mate with a female. Mating takes place with the male facing the reverse direction from the female, and with their ventral surfaces together. The apex of the abdomen of the male is applied to the base of the ovipositor of the female at the suture of the seventh sternite and nearly vertically below the caudal end of the propodeum. The contact may be almost instantaneous or may take several seconds. On one occasion the male was observed to tap the apex of the abdomen of the female while mating. The female extended her ovipositor as completely as if ovipositing. One female held the apex of her body up at an unusual angle for several seconds after mating. Frequently females stopped running at the touch of the antenna of a male.

Females evinced varying degrees of interest in eggs exposed to them. Efforts were made to supply the eggs in the condition most acceptable. It was anticipated that strictly fresh eggs would be most acceptable, and special care was taken to maintain this practice. However, the first egg attacked under observation contained a *Calendra* larva sufficiently developed to show it in motion. Often several eggs were exposed simultaneously. Sometimes as many as three females would pounce upon one egg and proceed to oviposit, ignoring the other eggs throughout the period of exposure. It was thought there might be some odor or substance left by the *Calendra* female upon or about the egg at time of oviposition, which determined the attractiveness of the egg to the parasite. Eggs exposed to the parasites in the egg cell in a small piece of the plant surely had the maximum possible odor from the female but were not attractive in any test made.

The adult female parasites were very demonstrative of their interest in *Calendra* eggs. There was the occasional business-like stab as quickly as the adult could attack the egg, sometimes all done in a few seconds. Frequently the successful stab was made only after much antennal tapping of the egg and much probing with the ovipositor in search for some portion of the chorion through which the ovipositor might be plunged. The adults often stand on tip-toe and try to apply the tip of the ovipositor vertically and at various other angles, sometimes using the tip of the abdomen to help improve the application of the ovipositor. When the thrust is successfully made, it may be deep, the body fairly close to the egg, or shallow, the ovipositor exposed equally with the legs, so that it was difficult to tell whether just six legs or six legs and the ovipositor were touching the egg. The inserted ovipositor may be held motionless or may be given various depths with one insertion, and may be given a shift in slant. The withdrawal of the ovipositor may be made with such ease that the observer cannot tell directly that there was a withdrawal. Sometimes the withdrawal is with apparent effort.

Other demonstrations of interest in the egg are contacts of the ventral surface of the abdomen on the egg, as if caressing the egg, walking on the egg and dropping the body almost, but not quite, to the egg, and contact of the mouth parts with the chorion, as if lapping up moisture or other substance from the surface of the egg. This behavior was at no time observed at a point of oviposition, as if utilizing an exudation of egg content.

THE EGG⁴

Several *Calendra* eggs, especially those of *C. maidis*, were attacked under observation by adult *Anaphoidea calendrae* and oviposition apparently was successfully accomplished. A few of these eggs were dissected immediately in distilled water, with stains, to make observations and avoid the distortion likely to

⁴ Dissection studies of the egg and of the larva were made by Alexander F. Bucholtz, Field Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology, U. S. Department of Agriculture, in the summer of 1928.

occur in the usual killing, fixing, infiltrating, and staining operations.

Acid fuchsin, eosin, methylin blue, and Langeron's cotton blue were tried, the last named giving the best results. It is a fixative stain. Its formula is: Acid phenol, 20 grams; acid lactic, 20 grams; glycerine, 40 grams; distilled water, 20 grams; cotton blue, a few grains on point of knife.

The first eggs thus dissected revealed nothing. Eggs were then dissected out from ovaries of recently issued *A. calendrae* adults. These have the caudal third scarcely half as thick as the cephalic two-thirds, the cephalic end tapering for about one-fifth of the total length. *A. calendrae* eggs were dissected from ovaries of several adults and from three host-eggs, with measurements as follows:

From a female 0.77 mm. long, reared through *C. minima* egg, one egg, 0.175 mm.; three, 0.192 mm. each; two, 0.227 mm. each; one, 0.262 mm. long.

From an unmeasured female, through *C. minima* egg, three eggs, 0.332 mm. long.

From a female 0.962 mm. long, through *C. maidis* egg from progeny from *C. minima* egg, one egg, 0.244 mm. long.

From a female 1.015 mm. long, through *C. maidis* egg from progeny from *C. minima* egg, one egg, 0.262 mm.; two, 0.280 mm. each; one, 0.297 mm.; one, 0.315 mm. long.

From a female 1.032 mm. long, through *C. maidis* egg from progeny from *C. minima* egg, one egg, 0.210 mm.; one, 0.227 mm.; two, 0.244 mm. long.

From a *C. maidis* host-egg, eight eggs, averaging 0.140 mm.

From a *C. maidis* host-egg, one egg, estimated 0.165 mm. long.

From a *C. maidis* host-egg, one egg 0.194 mm. long, oviposited by a female reared through *C. minima* egg.

A. calendrae eggs dissected out from the ovary and yet alive have a turgid appearance and the fine granular content reaches up to the egg-wall. The granular cytoplasm appears denser in the middle than elsewhere. This form is not definitely maintained in the earliest form observed in the host. Also, the size of the egg is reduced.

LARVÆ AS SEEN BY DISSECTION

Within the host eggs, *A. calendra* were observed to continue as eggs for 40 to 60 minutes after oviposition. Larvæ of the first instar were found 5 hours and 45 minutes after oviposition, at which time eight larvæ were removed from one *C. maidis* egg.

The first larval instar is represented by a long oval body, broadest about one-fourth or one-third of the body length back of the cephalic end, with a narrowed, tapering, appendiculate portion at caudal end. The alimentary tract appears definitely to terminate at base of this caudal portion. In this form, the larva bears at least four large setæ, about as long as the thickness of the body. These setæ do not stain. Measurements in the first instar are: One, 0.244 mm. long, from *C. maidis* egg, 28 hours after oviposition; one, 0.245 mm. in total length, the body proper being 0.175 mm., and the caudal appendage, 0.070 mm.

The second larval instar from 52 to 55 hours after oviposition. At this stage the larva has lost the caudal appendage and the setæ. A development at the cephalic end suggests mandibles slightly in anterior contour, but without significant adaptability to function. Measurements in the second instar, 52 to 55 hours after oviposition, from one *C. maidis* egg, are: One, 0.385 mm. long, 0.192 mm. wide; one, 0.385 mm. long, 0.157 mm. wide; one, 0.420 mm. long, 0.175 mm. wide; one, 0.367 mm. long, 0.157 mm. wide; and one, 0.350 mm. long, 0.175 mm. wide.

The third larval instar was studied from a series of larvæ removed from an egg of *C. venatus*, 100 to 103 hours after oviposition. They were nearly all short oval in form, being as thick as wide. They absorbed cotton blue stain very rapidly, obscuring anatomic characters. Measurements recorded for five are: 0.618 mm. long and 0.490 mm. wide; 0.635 mm. long, 0.367 mm. wide; 0.542 mm. long, 0.315 mm. wide; 0.517 mm. long, 0.437 mm. wide; and 0.507 mm. long, 0.350 mm. wide. One larva was in less compact form and distinctly revealed segmentation before staining, but the actual number of segments was not determined.

Hibernating larvæ dissected out of an egg believed to be *C. minima* were of the same compact oval-globular form of several dissected out of the *C. venatus* egg. It is assumed that these were also in the third instar. No molting was observed and no

exuviae were found. Hence the assumption that there are three instars is on the basis of the three types of larvæ observed.

Anaphoidea calendra eggs and larvæ found in dissected host-eggs indicate that reproduction in this species is not by polyembryony, and that the number of eggs found corresponds with records of issuance of adults.

LARVÆ AND PUPÆ AS SEEN WITHOUT DISSECTION

The accompanying table of host-eggs and observations of development of *Anaphoidea calendra* therein (table 2), is arranged arbitrarily in the order of lapse of time between possible parasitization and first recognition of the parasite larvæ, without any particular test having been made at short, regular intervals to discover the larvæ. The shortest time required in this series for development of larvæ, visible with no higher magnification than a pocket lens, was 64 hours, in *C. destructor* egg 24169. It is possible that, with care, larvæ would have been recognizable in all other eggs within this same period of time.

Pupæ were recognized almost at the moment of pupation in *destructor* egg 24169, 184 hours, 55 minutes after exposure of host-egg to parasites, and in *costipennis* egg 24168, 406 hours, 28 minutes after exposure. Larvæ were definitely recognized in these two eggs 20 hours, 35 minutes, and 12 hours, 30 minutes, respectively, prior to the recognition of pupæ. Pupæ in another *costipennis* egg were recognized 257 hours, 41 minutes after exposure and, so far as observations of larvæ were recorded, might have been pupæ for 116 hours. The pupæ observed in the shortest time after exposure were in a *parvula* egg, 169 hours, 27 minutes from exposure.

Periods of time from exposure of host-eggs to *A. calendra* adults to issuance of progeny adults ranged for the several species as follows: *C. destructor*, 307 hours, 40 minutes, to 379 hours, 4 minutes, 7 eggs; *costipennis*, 350 hours, 28 minutes, to 591 hours, 37 minutes, 2 eggs; *parvula*, 309 hours, 57 minutes, to 408 hours, 53 minutes, 2 eggs; *pertinax*, 303 hours, 30 minutes, to 408 hours, 6 eggs; ? *minima*, 336 hours, 40 minutes, 1 egg; *callosa*, 357 hours, 49 minutes, to 431 hours, 15 minutes, 6 eggs; *maidis*, 392 hours, 31 minutes, to 499 hours, 40 minutes, 2 eggs.

It is difficult to fix the causes for such variations in required time for development in different eggs of one species and of several species. Figure 1 visualizes the presence of variation for several groups on the same and on approximate dates, demonstrating that temperature is not entirely responsible. The host-species is not greatly the time controlling element. There appears to be no significant correlation between the number of individuals in the egg and the time of development.

The development of the parasites proceeds in this fashion, as seen with the aid of the hand lens: About 64 hours after oviposition, oval bodies, distinct as units from each other and from fat bodies, may be observed in the parasitized egg, even though the mandibles of the *Calendra* may be or may become prominent. As these oval larvæ grow, they take positions transverse or nearly so to the longitudinal axis of the egg, and surge back and forth in the spaces they have grown to occupy. No tendency was observed, in any normally filled egg, for them to change about.

When about to pupate, the larvæ change from transverse to longitudinal positions and the approach of pupation may thus be anticipated by several hours. As pupation takes place, the appendages become discernible before the heads or compound eyes. The compound eyes become discernible by their facets before any pigmentation has taken place. The color of the compound eyes progresses from white, through pale salmon, to salmon red, brilliant red, brown, and black. The bodies remain white from pupation until about 72 hours before issuance, and because of the brilliant red compound eyes they are strikingly beautiful at this pre-blackening moment. The black pigment first appears in the coxæ and ventral extensions of the eighth tergite, the armature for the ovipositor, then in pronotum and face, the general appearance of the egg passing through a slaty blue for a portion of a day before the pupa becomes black. Unconsumed egg contents, when present, are white, contrasting strikingly with the black pupæ.

METHODS USED IN PROPAGATION

The adults of *Anaphoidea calendra* are so small that special methods of handling them are necessary. A large sheet of

TABLE 2

SPECIES OF *Calendra* EGGS, WITH NUMBER OF HOURS WITHIN WHICH THE SEVERAL GROUPS OF PARASITES WERE OBSERVED AS LARVÆ, AS PUPÆ, AND AS ADULTS, RESPECTIVELY, AND NUMBER (BY SEX) OF RESULTING ADULTS.

Species of host-egg	Time (hours) between exposure and first observation of—					
	Larva	Pupa	Adult,	♂	♀	?
<i>destructor</i>	64	185	352	1	7	
<i>callosa</i>	76	243	360	22	1	
<i>callosa</i>	78	245	386	9	0	
<i>pertinax</i>	80	193	359	2	19	
<i>destructor</i>	81	194	312	13	1	
<i>pertinax</i>	95	187	304	0	8	
<i>pertinax</i>	95		327	1	5	
<i>callosa</i>	97	320	432	1	5	
<i>pertinax</i>	98	191	309	1	7	
<i>parvula</i>	103	170	310	1	9	
<i>costipennis</i>	113	258	351	1	11	
<i>callosa</i>	118	285	400	10	0	
<i>callosa</i>	118	285	402	8	0	
<i>destructor</i>	122	287	308	3	6	1
<i>destructor</i>	122	287	380	6	2	
<i>maidis</i>	123	217	393	1	7	
<i>pertinax</i>	124	270	334	1	7	
<i>destructor</i>	124	222	335	1	6	
<i>destructor</i>	144	192	338	0	12	4
<i>pertinax</i>	144	239	408	0	0	5
<i>costipennis</i>	161	407	592	1	11	
<i>destructor</i>	169	214	330	8	0	
<i>callosa</i>	172	222	358	1	9	
<i>maidis</i>	211	379	500	0	10	
<i>parvula</i>	218	318	409	2	8	3
? <i>minima</i>		262	337	0	4	3

white paper is very desirable as a working background. Transparent containers of small size are indispensable. Opaque containers are impracticable, both as places in which the tiny creatures may be lost, alive or dead, and as receptacles from which they may escape with the admission of light or with the draft produced by the removal of lid or cork.

The type of container used in these studies was a culture tube about 35 mm. deep and 9 mm. in diameter, having a rounded bottom, and stopped with tightly bunched cotton. Each tube was marked with a number on a small bit of adhesive tape at rim, outside. The host-egg was placed in the tube before possibility of issuance of adults. After the adults issued, they were

watched in many instances until matings had taken place, certainly until it was known that both sexes were represented, when one or more *Calendra* eggs were placed in the tube by use of a delicate camel's-hair brush.

The adults are usually attracted to light fairly promptly. When necessary, the tube was darkened except at the bottom, to induce the adults to leave the mouth of the tube. Usually this was not necessary, as they would go toward the bottom when it was placed toward the source of light, either natural or artificial. While they were near the bottom, the stopper was removed, the egg or eggs were introduced, and the stopper replaced.

Several schemes were tried for supplying the eggs on a service tray composed of a longitudinal section of a plant stem, but this was too good a perch for the adults and removal was too often delayed by reason of the adults resting on it. Another difficulty was introduced when such device was placed in the tube. When the device contained moisture, this was deposited on the inside of the tube, thus reducing visibility and causing fatal adhesions of adults to the glass.

Further regulation of moisture within the tubes was attained by inserting bits of corn pith or dry or damp bits of white blotter, as conditions demanded. Atmospheric conditions within the tubes had to be regulated for the host-eggs as well as for the adult parasites.

Once under way, and with the earthen cages for host-eggs placed conveniently close to the parasite tubes, a new egg could be placed in the tube and the exposed egg removed by one opening of the tube, the brush and the stopper replaced, with a minimum chance for the escape of the adults. Even with the utmost care, sometimes the entire set of adults, more frequently a fraction of the set, would fly or jump out. Empty culture tubes may be placed vertically over one after another of the adults as expeditiously as possible, and when no more remain in sight, the captured adults may be returned to one tube, one at a time, by putting the mouths of the tubes together and holding the bottom of the one containing the greater number of adults to the

light until all are in one tube. These may be conveyed to the tube containing the host-egg, or the egg may more readily be transferred to the tube containing the adults.

The tubes containing parasites were laid on damp soil in closed tin salve boxes over night, with satisfactory results. Desiccation tendencies are generally forestalled, both in relation to parasites and to host-eggs, by this method of handling.

Eggs that had been exposed to adult parasites were kept in the Calendra egg cage, consisting of damp soil compactly pressed into the tin ointment box for about two-thirds the depth of the box, the eggs being given individual depressions representing definite sub-cage numbers, until the eggs hatched or showed parasite development.

Eggs revealing parasite development were given a new cage number, with records written from the standpoint of the parasite, and were kept on damp soil until the parasite pupæ began to blacken, when they were placed in the culture tubes with cotton stoppers, still on damp soil in tin ointment boxes. As the adults issued, the cycle of method was continued.